



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

10

April 13, 1998

MEMORANDUM

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

SUBJECT: Pirimiphos-methyl. PP#6F4740. Residue Chemistry Issues to be Presented to the Metabolism Assessment Review Committee. Chemical No. 108102. DP Barcode No. D228695.

FROM: Jerry B. Stokes, Chemist  
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THROUGH: Susan V. Hummel, Branch Senior Scientist  
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TO: Metabolism Assessment Review Committee  
George Krammer, Exec. Sec.  
Health Effects Division (7509C)

**TOLERANCES:** Tolerances are established (40 CFR §180.409) for residues of Pirimiphos-methyl and its metabolite O-[2-ethylamino-6-methyl-pyrimidin-4-yl) O,O-dimethyl phosphorothioate (Compound A; See Figure 1) and, in free and conjugated form, the metabolites 2-diethylamino-6-methyl-pyrimidin-4-ol (Compound C), 2-ethylamino-6-methyl-pyrimidin-4-ol (Compound D), and 2-amino-6-methyl-pyrimidin-4-ol (Compound E) in corn (8.0 ppm), sorghum grain (8.0 ppm), kiwifruit (5.0 ppm), eggs (0.5 ppm), milk fat [3.0 ppm; 0.1 (N) in whole milk]; fat of cattle, goats, hogs, horses, poultry, and sheep at 0.2 ppm; kidney and liver of cattle, goats, hogs, and sheep at 2.0 ppm; meat and meat byproducts of cattle, goats, hogs, horses, and sheep at 0.2 ppm; and meat and meat byproducts of poultry at 2.0 ppm.

Food and feed additive tolerances have been established under 40 CFR §185.4950 and §186.4950 for residues in corn milling fractions (except flour) and sorghum milling fractions (except flour) at 40 ppm and in 40 CFR §185.4950 for residues in corn oil at 88 ppm. The



tolerances for residues in corn and sorghum grain were established for postharvest application to stored grain.

Tolerances for pirimiphos-methyl residues were reassessed in conjunction with the preparation of the residue chemistry chapter of the HED reregistration eligibility decision document (RED) chapter. Tolerances for residues in sorghum and corn grain should be increased to 20 ppm, which leads to an increase in the corn oil tolerance to 160 ppm, and the need for a tolerance for residues in aspirated grain fractions at 50 ppm.

**MODE OF ACTION:** Cholinesterase inhibitor

**REQUEST TO THE COMMITTEE:**

**CEB2 requests the MARC to determine the pirimiphos-methyl residues of concern and what residues should be included in the tolerance expression and what residues should be considered in risk assessments.**

**DETAILED CONSIDERATIONS**

### **Corn Metabolism**

Corn grain was treated with [2-<sup>14</sup>C]pirimiphos-methyl to simulate the registered application to grains prior to placement in storage. Three successive applications were made at 28 mg ai/600 g of grain per application, equivalent to 2.8 lb ai/30 tons (6X the maximum registered rate). Grain samples were collected on the day of application and after 12 and 24 weeks of storage at ambient temperature in a desiccator. Total radioactive residues in the zero day corn samples ranged from 86.8 to 110.9 ppm. At 12 weeks after treatment (Group 2) radioactivity had decreased to 50.4-53.3 ppm and after 24-weeks (Group 3) total radioactive residues were 43.1-45.3 ppm.

Activity in corn grain extracts was identified via co-elution with non-radiolabeled standards using high performance liquid chromatography (HPLC) with a gradient solvent system. Identification of pirimiphos-methyl metabolites (SEE Figure 1.) was confirmed using thin layer chromatography (TLC). The major residue in corn grain treated postharvest with [<sup>14</sup>C]pirimiphos-methyl was the parent, pirimiphos-methyl *per se*, which constituted >96% of the TRR in zero-time samples and 64-67 %TRR in 12 and 24 week samples. Metabolites A and C were the only metabolites detected in zero day samples, each at ≤2.4% of the TRR. Metabolites A and C were found in 12 week samples at up to 10.3 %TRR, with metabolites D and E/F present at up to 3.2 %TRR. Unidentified radioactivity in 12-week samples totaled 14.5 %TRR, but with no one component at >2 %TRR. In 24-week samples, metabolite C constituted up to 22 %TRR, metabolite D was identified at up to 8.6 %TRR, and metabolites E/F were present at ≤0.2 %TRR.

The principal residues in stored grain consist of the parent, pirimiphos-methyl, and its metabolite O-[2-ethylamino-6-methyl-pyrimidin-4-yl] O,O-dimethyl phosphorothioate (Metabolite A, R36341).

### Total radioactive residues (TRR)

**TABLE 1. Total Radioactive Residues in Corn Grain.**

Sampling interval (weeks)	TRR (ppm) <sup>a</sup>
0 (Group 2)	94.7 93.0 86.8 Average = 91.5
0 (Group 3)	93.8 110.9 88.8 Average = 97.8
12 (Group 2)	50.4 53.3 50.7 Average = 51.5 <i>50.0 50.8 50.3</i>
24 (Group 3)	43.1 45.3 43.8 Average = 44.1 <i>38.0 46.8 53.5</i>

<sup>a</sup> Sum of radioactivity in first MeOH extract and extracted grain; values in *italics* are the results from combustion LSS of whole grain samples, not yet extracted.

**TABLE 2. Identification/Characterization of [<sup>14</sup>C]pirimiphos-methyl residues in corn grain.**

Sampling interval	Zero time	12 weeks	24 weeks
Metabolite/fraction	Percent TRR in matrix (ppm in parentheses)		
Pirimiphos-methyl	96.2-97.3 (83.3-92.0)	64.3-72.7 (32.6-36.7)	64.4-67.4 (27.5-30.2)
Compound A	1.5-2.4 (1.4-2.1)	2.7-10.3 (1.4-5.2)	ND <sup>a</sup>
Compound C	ND-0.9 (ND-0.9)	5.3-10.2 (2.7-5.2)	19.3-22.1 (8.7-9.4)
Compound D	ND	0.1-3.2 (0.05-1.7)	8.1-8.6 (3.5-3.9)
Compound E/F	ND	0.8-2.5 (0.5-1.2)	<0.1-0.2 (0.02-0.07)
HPLC unknowns <sup>b</sup>	0.1 (0.05-0.1)	4.8-14.5 (2.4-7.4)	0.2-0.3 (0.07-0.14)
Insoluble	0.4-0.5 (0.4-0.5)	3.6-3.8 (1.8-2.0)	4.7-5.0 (2.3-4.7)
Total identified/characterized	99.2-99.9	99.3-99.5	99.5-100

<sup>a</sup> ND = Not detected. <sup>b</sup> Numerous unknowns, each constituting <5 %TRR.

Figure 1. Chemical names and molecular structures of pirimiphos-methyl and its metabolites in plants and animals.

Common Name	Structure
<p>O-(2-diethylamino-6-methylpyrimidin-4-yl)-O,O-dimethyl phosphorothioate</p> <p><b>Pirimiphos-methyl</b></p> <p>(Compound III: pirimiphos oxon, P=O)</p>	
<p>O-(2-ethylamino-6-methylpyrimidin-4-yl)-O,O-dimethyl phosphorothioate</p> <p><b>Compound A</b> <b>R36341, des-ethyl pirimiphos-methyl, DPM</b></p>	
<p>2-diethylamino-6-methyl-pyrimidin-4-ol</p> <p><b>Compound C</b> <b>R46382</b></p>	
<p>2-ethylamino-6-methyl-pyrimidin-4-ol</p> <p><b>Compound D</b> <b>R35510</b></p>	
<p>2-amino-6-methyl-pyrimidin-4-ol</p> <p><b>Compound E</b> <b>R4039</b></p>	
<p>2-hydroxy-6-methyl-pyrimidin-4-ol</p> <p><b>Compound F</b></p>	

TABLE 3. Extraction of [ $^{14}\text{C}$ ]pirimiphos-methyl residues from Corn Grain.

Fraction	% TRR	ppm	Analysis
<b>Corn Grain (Group 2) Zero Time (86.8-94.7 ppm)</b>			
MeOH I	99.0-99.3	85.8-94.0	HPLC: Pirim-methyl 96.2-97.3% 83.3-92.0 ppm Metabolites: A: 1.5-2.4%, 1.4-2.1 ppm C: ND-0.7%, ND-0.7 ppm
Insoluble	0.69-0.95	0.75-0.96	Acid hydrolysis: 2 M HCl 50% in MeOH, 20 min; hexane partition, evaporate, reconst in MeOH
MeOH II	0.21-0.33	0.23-0.33	HPLC: Metabolites: C: 0.2%, 0.17-0.28 ppm Unknowns: 0.1%, 0.05-0.11 ppm
Insoluble II	0.36-0.48	0.40-0.48	Further analysis did not yield additional information
<b>Corn Grain (Group 2) 12 Weeks (50.4-53.3 ppm)</b>			
MeOH I	94.1-94.6	47.6-50.2	HPLC: Pirim-methyl 64.3-72.7% 32.6-36.7 ppm Metabolites: A: 2.7-10.3%; 1.4-5.2 ppm C: 4.4-9.2%; 2.2-4.7 ppm D: 0-3.1%, 0-1.6 ppm E/F: 0.7-2.4%, 0.4-1.2 ppm Unknowns: 4.5-14.4%, 2.3-7.3 ppm
Insoluble I	5.41-5.90	2.74-3.15	Acid hydrolysis: 2 M HCl 50% in MeOH, 20 min; hexane partition, evaporate, reconst in MeOH
MeOH II	1.3-1.7	0.64-0.90	HPLC: Metabolites: C: 0.9-1.1%, 0.46-0.58 ppm D: 0.1%, 0.50-0.11 ppm E/F: 0.1-0.2%, 0.03-0.08 ppm Unknowns: 0.1-0.3%, 0.05-0.13 ppm
Insoluble II	3.6-3.8	1.81-2.02	Further analysis did not yield additional information
<b>Corn Grain (Group 3) 24 Weeks (43.1-45.3 ppm)</b>			
MeOH I	93.6-94.1	40.1-41.9	HPLC: Pirim-methyl 64.4-67.4% 27.5-30.2 ppm Metabolites: C: 18.7-21.5%, 8.4-9.2 ppm D: 8.0-8.4%, 3.5-3.8 ppm
Insoluble I	5.92-6.40	3.02-3.43	Acid hydrolysis: 2 M HCl 50% in MeOH, 20 min; hexane partition, evaporate, reconst in MeOH
MeOH II	0.95-1.10	0.48-0.56	HPLC: Metabolites: C: 0.5-0.8%, 0.25-0.38 ppm D: 0.1-0.2%, 0.05-0.09 ppm E/F: 0.1-0.2%, 0.02-0.07 ppm Unknowns: 0.2-0.3%, 0.07-0.14 ppm
Insoluble II	4.7-5.0	2.3-2.7	Further analysis did not yield additional information

Comments for past Chemistry Branch review dated March 29, 1979 (R. Perfetti)**Metabolism of pirimiphos-methyl in stored wheat, rice, and peanuts.**

The grains were treated with 4 and 8 ppm C14 pirimiphos and stored in dark at 25 C and constant humidity for upto 32 weeks. After this time, the extractable residues in low moisture grain consisted of 79 to 86% parent, and 9 to 11% of the 3 pyrimidines (compounds C, D, and E), 5 to 10% Compound A, and other unidentified products. Residues in high moisture grain were 40% parent, 50 to 55% of 3 pyrimidines C, D, and E, 9 to 15% A, and other unidentified products.

A study of pirimiphos-methyl in waters (pond, river, sterile, and rice water) showed degradation by hydrolysis to form compound C and traces amounts of "oxy" pirimiphos-methyl (Compound III, structure not shown, but is the oxon of pirimiphos-methyl) were tentatively identified. Hydrolysis is accelerated by the presence of light.

Peanuts in the shell were treated with 35 ppm C14 pirimiphos-methyl and stored in metal drums for up to 91 days. The extractable residue (86 to 89% of applied radioactivity) in peanut hulls consisted of parent 75%, Compound C 2%, Compound A 2.3%, and "oxy" pirimiphos-methyl 0.4%, and <20% unidentified. The extractable residue in peanut nutmeat consisted of parent 29%, while the remaining 79% was not identified. Residues identified in peanut hulls consisted of parent, Compound A, Compounds C, D, and E, and "oxy" pirimiphos-methyl (compound III) in decreasing order. **Note:** Compound III was not observed in stored wheat or rice grains.

The above studies indicate that the pathway is mainly hydrolytic in nature in stored wheat and rice grains, and oilseed peanuts.

Additional data for peanuts using C14 pirimiphos-methyl was reviewed (02/14/80, J. Worthington). Stored peanuts were treated at 31 ppm and stored for 6 months. The hulls contained residues as high as 168 ppm. The nature of the residues in/on the hulls were not investigated. The seed coat (ca. 3% wgt of kernel) contained 82% of the activity. Residues consisted of parent (11 to 26%), Compound C (23 to 37%), and unidentified (37 to 58%). Hydrolysis of these unidentified residues revealed 5% parent and 21% Compound C. Extraction (ca. 90% of total residue) of the seed kernel showed the residues which reached a high of 0.6 ppm was parent. Residues on the kernel was identified as parent (33%), as pyrimidinols (45%), polar conjugates (12%), and unidentified (10%).

Residue Analytical Methods

Residues of pirimiphos-methyl and its metabolite des-ethyl pirimiphos-methyl are determined by GLC equipped with a phosphorus detection using Method CSI-011 (versions 04 and 06). This method is a modification of the method currently listed in PAM, Vol II. LOQ's are 0.05- 0.1

ppm in grains (both parent and des-ethyl compound) and some processed products, and slightly higher for others (0.20 - 0.25 ppm). This PAM method can be used to analyze for the "oxy" pirimiphos-methyl without any extraction or GLC changes. The oxon has slightly longer retention time. Detection of the pyrimidinols requires a different method since these compounds are not phosphorus containing.

## **CODEX**

Codex regulates the parent pirimiphos-methyl only. Pirimiphos-methyl is used on many crops in other countries such as fresh fruits and vegetables, in addition to grains/oilseeds. The oxon is not included a residue of concern for any of these crops. Likewise the pyrimidinols are not included as residue of concern.

## **Animal Metabolism:**

### **Ruminants (Goat Metabolism)**

Goats were dosed for 7 days with [2-<sup>14</sup>C]pirimiphos-methyl at 10 and 50 ppm and the dose levels represent approximately 0.5x and 2.6x the maximum theoretical dietary intake of 19 ppm by beef cattle. Total radioactive residues (TRRs) plateaued in milk by approximately day 4. Maximum milk TRRs were 0.102 ppm for the low-dose and 0.302 ppm for the high-dose. Radioactivity in tissues (high-dose) ranged from 0.008-0.013 ppm in muscle to 0.190-0.198 ppm in liver.

Radioactive residues in selected soluble and hydrolyzed fractions were analyzed using HPLC, with confirmation via TLC. Standard compounds consisted of the parent, pirimiphos-methyl, the metabolite des-ethyl pirimiphos-methyl (Compound A), and four hydroxy-pyrimidine metabolites (Compounds C, D, E, and F) (See Figure 1.).

Metabolism of pirimiphos-methyl in goats involves N-dealkylation of the parent to Compound A or hydrolysis of the O-P bond to Compound C. Hydrolysis of Compound A or dealkylation of Compound C could yield Compound D. Compound D is dealkylated to Compound E, which subsequently is deaminated to Compound F. It is likely that the hydroxypyrimidine metabolites C, D, E, and F would be subject to further breakdown by natural pyrimidine catabolic pathways and incorporation into biological components.

Pirimiphos-methyl and Compound A (the only organophosphate metabolite), and four hydroxy-pyrimidine metabolites are the residues in meat, milk and meat byproducts of ruminants. The only cholinesterase-inhibiting metabolites identified in ruminant tissues and milk are the parent, pirimiphos-methyl, and Compound A.

**Ruminants: Total radioactive residues (TRR)****Table 4. Total [ $^{14}\text{C}$ ]Pirimiphos-methyl Residues in Milk (afternoon milking).<sup>a</sup>**

Treatment day	Milk - Low dose (10 ppm)		Milk - High dose (50 ppm)	
	Goat #3	Goat #4	Goat #5	Goat #6
1	0.100	0.045	0.228	0.217
2	0.097	0.057	0.257	0.194
3	0.093	0.056	0.297	0.209
4	0.100	0.061	0.295	0.216
5	0.102	0.057	0.298	0.215
6	0.102	0.060	0.302	0.222
7	0.100	0.062	0.298	0.195

<sup>a</sup> $^{14}\text{C}$ -pirimiphos-methyl equivalents, ppm.**Table 5. Total [ $^{14}\text{C}$ ]Pirimiphos-methyl Residues in Ruminant Tissues.**

Tissue	Total radioactive residue (ppm)			
	Low dose (10 ppm)		High dose (50 ppm)	
	Goat #3	Goat #4	Goat #5	Goat #6
Blood	0.009	0.007	0.023	0.016
Heart	0.010	0.007	0.027	0.019
Kidney	0.044	0.050	0.118	0.079
Liver	0.083	0.045	0.198	0.190
Hindquarter	0.006	0.005	0.013	0.008
Tenderloin	0.006	0.004	0.013	0.008
Back fat	0.022	0.001	0.032	0.023
Omental fat	0.021	0.019	0.057	0.080

**Table 6. Extraction of Radioactive Residues from Ruminant Milk and Tissues (High-Dose Goat).**

Fraction	% TRR	ppm	Analysis
<b>Milk - Day 4, 0.295 ppm</b>			
ACN:H <sub>2</sub> O	72.1	0.213	Phase separation
ACN	63.0	0.186	Pirim-methyl: 16.7%, 0.049 ppm C/D: 9.0%, 0.027 ppm E/F: 36.1%, 0.106 ppm
H <sub>2</sub> O	NA <sup>a</sup>	NA	Hexane partition
H <sub>2</sub> O	6.9	0.020	Unknowns 6.9%, 0.020 ppm
Hexane	2.1	0.006	Not analyzed
Insoluble	27.9	0.082	Acid hydrolysis, 2 M HCl 50% in MeOH, hexane partition; MeOH extraction
MeOH I	14.0	0.041	Unknowns: 14.0%, 0.041 ppm
Insoluble	13.7	0.040	No further analysis
<b>Liver; 0.198 ppm</b>			
ACN:H <sub>2</sub> O	20.1	0.040	Phase separation
ACN	16.1	0.032	Pirim-methyl 7.1%, 0.014 ppm Unknowns 9.1%, 0.018 ppm
H <sub>2</sub> O	NA	NA	
H <sub>2</sub> O			1.37%, 0.003 ppm
Hexane			2.61%, 0.005 ppm
Insoluble	79.9	0.158	Acid hydrolysis, 2 M HCl 50% in MeOH, hexane partition; MeOH extraction
MeOH I	17.4	0.034	Unknowns: 17.4%, 0.034 ppm
Insoluble	NA	NA	Acid hydrolysis, 2 M HCl reflux 1 hr; MeOH extraction
HCl:MeOH	43.3	0.086	Unknowns: 43.3%, 0.086
Insoluble	17.4	0.036	No further analysis

Fraction	% TRR	ppm	Analysis
<b>Kidney, 0.118 ppm</b>			
ACN:H <sub>2</sub> O	61.5	0.073	Phase separation
ACN	48.9	0.058	Metabolites: C: 6.6%, 0.008 ppm E/F: 32.2%, 0.038 ppm Unknowns 10.1%, 0.012 ppm
H <sub>2</sub> O	NA	NA	Hexane partition
H <sub>2</sub> O	11.0	0.013	No results reported
Hexane	1.6	0.002	Not analyzed
Insoluble	38.5	0.045	Acid hydrolysis, 2 M HCl 50% in MeOH, hexane partition; MeOH extraction
MeOH I	24.7	0.029	Unknowns: 23.1%, 0.029 ppm
Insoluble	NA	NA	Acid hydrolysis, 2 M HCl, reflux 1 hr; MeOH extraction
Hcl:MeOH	7.6	0.009	Unknowns: 7.6%, 0.009 ppm
Insoluble	4.2	0.005	No further analysis
<b>Tenderloin muscle, 0.013 ppm</b>			
ACN:H <sub>2</sub> O	75.8	0.010	Phase separation
ACN	54.2	0.007	Unknowns 54.2%, 0.007 ppm
H <sub>2</sub> O	NA	NA	Hexane partition
H <sub>2</sub> O			7.58%, 0.001 ppm
Hexane			14.1%, 0.002 ppm
Insoluble	24.2	0.003	Acid hydrolysis, 2 M HCl 50% in MeOH, hexane partition; MeOH extraction
Hcl:MeOH	4.1	0.001	No further analysis
Insoluble	NA	NA	Filter through scintered glass
Filtrate	13.9	0.002	No further analysis
Insoluble	6.2	0.001	No further analysis

Fraction	% TRR	ppm	Analysis
<b>Fat (omental), 0.057 ppm</b>			
Hexane	52.2	0.030	Partition with ACN
ACN	52.0	0.030	Pirim-methyl: 39.1%, 0.023 ppm A: 12.9%, 0.007 ppm
Hexane	0.3	<0.001	Not analyzed
Insoluble	47.8	0.027	Solvent extraction DCM, hexane, ACN
ACN II	24.6	0.014	No further analysis
Insoluble	11.1	0.006	

<sup>a</sup> NA = Sample not analyzed for radioactivity.

In summary, pirimiphos-methyl is metabolized by N-dealkylation to Compound A or by cleavage of the O-P bond to Compound C. Compound C is dealkylated to Compound D, which then may be metabolized to Compounds E and F. Compounds E and F are hydroxy-pyrimidines that could be metabolized through the pyrimidine catabolic pathway to natural products.

The residues in ruminants are the parent compound and Compound A, the only other organophosphate compound detected, and the four identified hydroxy-pyrimidine metabolites, in free and conjugated form.

**Table 7. [<sup>14</sup>C]Pirimiphos-methyl Metabolite Characterization/Identification in Ruminant Milk and Tissues.**

Matrix Metabolite/ fraction	Milk day 4, p.m. (0.295 ppm)	Liver (0.198 ppm)	Kidney (0.118 ppm)	Tenderloin muscle (0.013 ppm)	Omental fat (0.057 ppm)
	Percent TRR in matrix (ppm in parentheses)				
Pirimiphos-methyl	16.7 (0.049)	7.1 (0.014)	ND <sup>a</sup>	ND	39.1 (0.023)
Compound A	ND	ND	ND	ND	12.9 (0.007)
Compound C	9.0 (0.027)	ND	6.6 (0.008)	ND	ND
Compound D		ND	ND	ND	ND
Compounds E/F	36.1 (0.106)	ND	32.2 (0.038)	ND	ND
Total Identified	61.8 (0.182)	7.1 (0.014)	38.8 (0.046)	0.00	52.0 (0.030)
HPLC unknowns <sup>b</sup>	20.9 (0.062)	69.8 (0.138)	40.8 (0.050)	54.2 (0.007)	ND
Other <sup>c</sup>	15.8 (0.046)	21.4 (0.044)	5.8 (0.007)	45.9 (0.006)	36.0 (0.020)
Total partially Characterized	36.7 (0.108)	91.2 (0.182)	46.6 (0.057)	100.1 (0.013)	36.0 (0.020)
Total identified + partially characterized	98.5 (0.291)	98.3 (0.195)	85.4 (0.101)	100.1 (0.013)	88.0 (0.050)

<sup>a</sup> ND = Not detected.<sup>b</sup> Total of unknown HPLC peaks/areas each at <5% of TRR.<sup>c</sup> Total of numerous fractions, each considered characterized to some extent and accounting for <10% of TRR and/or <0.05 ppm of radioactivity.

### Poultry Metabolism

Hens were dosed with [2-<sup>14</sup>C]pirimiphos-methyl at rates of 12 and 68 ppm for 7 days prior to sacrifice. The dose levels represent approximately 0.75x and 4.3x the maximum theoretical dietary intake of 16 ppm. Total radioactive residues (TRRs) plateaued in egg white by Day 3.

Radioactive residues in poultry tissues and eggs were adequately identified or characterized. The metabolic pathway in poultry is assumed to be similar to that in ruminants, although the distribution of radioactivity between tissue types (i.e., muscle, liver and fat) is somewhat different. Identified residues include the parent pirimiphos-methyl, the des-ethyl metabolite, and four hydroxy-pyrimidine metabolites.

#### **Total radioactive residues (TRRs)**

**Table 8. Total [<sup>14</sup>C]Pirimiphos-methyl Residues in Eggs.**

Treatment day	Egg white TRR (ppm)		Egg yolk TRR (ppm)	
	12 ppm	68 ppm	12 ppm	68 ppm
1	0.024	0.022	0.006	0.005
2	0.049	0.298	0.013	0.080
3	0.059	0.414	0.024	0.153
4	0.061	0.364	0.034	0.187
5	0.063	0.386	0.048	0.261
6	0.054	0.301	0.056	0.310
7	0.040	0.324	0.058	0.543

**Table 9. Total [<sup>14</sup>C]Pirimiphos-methyl Residues in Edible Tissues from Hens.**

Tissue	Total radioactive residue (ppm)	
	12 ppm	68 ppm
Blood	0.056	0.425
Liver	0.033	0.210
Muscle	0.145	0.634
Kidney	0.045	0.335
Fat	0.028	0.336
Skin	0.028	0.309

**Table 10. Extraction of Radioactive Residues from Eggs.**

Fraction	% TRR	ppm	Analysis
<b>Egg white - Day 4, 0.362 ppm</b>			
MeOH I	90.0	0.326	Pirim-methyl 3.4%, 0.012 ppm A: 0.7%, 0.003 ppm; C: 0.7%, 0.003 ppm; D: 1.4%, 0.005 ppm; E/F: 63.2%, 0.229 ppm Unknowns 20.6%, 0.074 ppm
Insoluble I	9.98	0.036	HCL hydrolysis (2 M HCl, 50% in MeOH, 10 min) Hexane partition
MeOH II	3.88	0.014	F: 3.1%, 0.011 ppm Unknowns 0.76%, 0.003 ppm
Insoluble II	3.31	0.012	Acid hydrolysis (2 M HCl, 10 min) yielded 1.65%, 0.006 ppm
<b>Egg yolk - Day 4; 0.186 ppm</b>			
Hexane:EtOH	NA <sup>a</sup>	NA	Partitioned with H <sub>2</sub> O
EtOH:H <sub>2</sub> O	33.2	0.062	C: 4.09%, 0.008 ppm D: 22.1%, 0.041 E/F: 7.01%, 0.013
Hexane I	NA	NA	Extract with ACN
ACN	12.8	0.024	Pirim-methyl 12.1%, 0.023 ppm C: 0.4%, 0.001 ppm E/F: 0.3%, 0.001 ppm
Hexane II	2.91	0.005	No further analysis
Insoluble I	NR	NR	Extract with MeOH
MeOH	39.4	0.073	Oily fraction could not be analyzed by HPLC or TLC
MeOH:H <sub>2</sub> O	4.24	0.008	Not analyzed
Acetone	0.72	0.001	
Insoluble	6.75	0.013	Acid hydrolysis (HCl, 50% in MeOH, 10 min); HPLC of hydrolysate in MeOH: 1% of TRR unknowns

<sup>a</sup> NR = not determined/reported

**Table 11. Extraction of Radioactive Residues from Hen Tissues.**

Fraction	% TRR	ppm	Analysis
<b>Liver; 0.210 ppm</b>			
MeOH:H <sub>2</sub> O	38.1	0.080	Hexane partition
MeOH:H <sub>2</sub> O	34.6	0.073	Pirim-methyl 1.52%, 0.003 ppm A: 1.21%, 0.003 ppm; C: 0.83%, 0.002 ppm D: 14.2%, 0.030 ppm; E/F 12.7%, 0.0027 ppm Unknowns 4.1%, 0.008 ppm
Hexane	3.56	0.007	ACN partition: hexane 0.94%, 0.002 ppm ACN 2.6%, 0.006 ppm
Insoluble	61.9	0.130	Acid hydrolysis: 2 M HCl, 50% in MeOH, cleanup, evaporate, take up in MeOH
MeOH	3.96	0.008	All unknowns
Insoluble	57.6	0.121	Acid hydrolysis: 2 M HCl, extract with MeOH
cl:MeOH	18.6	0.039	All unknowns
Insoluble	32.9	0.069	No further analysis
<b>Muscle, 0.634 ppm</b>			
MeOH:H <sub>2</sub> O	82.9	0.526	Hexane partition
MeOH:H <sub>2</sub> O	81.6	0.517	Pirim-methyl 3.02%, 0.019 ppm A: 1.0%, 0.006 ppm; C: 3.1%, 0.020 ppm D: 45.2%, 0.287; E/F: 25.6%, 0.162 ppm Unknowns 3.59%, 0.023 ppm
Hexane	1.31	0.008	ACN partition: hexane 0.74%, 0.005 ppm ACN 0.57%, 0.004 ppm
Insoluble	17.1	0.108	Acid hydrolysis (2 M HCl in MeOH)
HCl:MeOH	7.89	0.050	Hexane partition
MeOH	7.26	0.046	Pirim-methyl 0.9%, 0.006 ppm F: 5.7%, 0.036 ppm Unknowns: 0.6%, 0.003 ppm
Insoluble	9.15	0.058	Acid hydrolysis (2 M HCl, 1 hr reflux)
Hcl:MeOH	5.68	0.036	All Unknowns
Insoluble	2.21	0.014	No further analysis

**Table 11. Extraction of Radioactive Residues from Hen Tissues.**

Fraction	% TRR	ppm	Analysis
<b>Fat, 0.336 ppm</b>			
Hexane	93.4	0.314	Partition with ACN
ACN	91.4	0.307	P-methyl 85.4%, 0.287 ppm A: 6.03%, 0.019 ppm E: 10.9%, 0.033 ppm
Hexane	2.07	0.007	No further analysis
Insoluble	6.56	0.022	No further analysis
<b>Skin, 0.309 ppm</b>			
DCM	42.0	0.130	Partition with hexane, ACN
ACN	36.7	0.113	Pirim-methyl 31.0%, 0.095 ppm A: 2.46%, 0.008 ppm C: 2.46%, 0.008 ppm Unknowns: 0.77%, 0.002 ppm
Hexane	5.35	0.017	Not analyzed
Insoluble	58.0	0.179	Acid hydrolysis (I) 2 M HCl, 50% in MeOH (II) 2 M HCl, reflux 1 hr Hydrolysates extracted with MeOH
MeOH I	25.3	0.078	A: 0.6%, 0.002 ppm C: 13.5, 0.042 ppm D: 1.4%, 0.004 ppm E/F: 3.4%, 0.011 ppm Unknowns: 6.4%, 0.020 ppm
MeOH II	20.1	0.062	C: 9.2, 0.028 ppm Unknowns: 10.9%, 0.034 ppm
Insoluble	7.82	0.024	Not further analyzed

The metabolic pathway in hens appears to be similar to that in cattle: pirimiphos-methyl is metabolized by N-dealkylation to Compound A or by cleavage of the O-P bond to Compound C. Compound C is dealkylated to Compound D, which then may be metabolized to Compounds E and F. Compounds E and F are hydroxypyrimidines that could be metabolized through the pyrimidine catabolic pathway to natural products.

Although the metabolites identified in both hen and ruminant tissues/milk/eggs are the same, it is interesting to note that total radioactivity in ruminant muscle was very low, while radioactivity in hen muscle was relatively high, exceeding that of the liver. The results of the two studies suggest that pirimiphos-methyl is incorporated into natural products to a greater extent in the ruminant than in the hen.

**Table 12. [<sup>14</sup>C]Pirimiphos-methyl Metabolite Characterization/Identification in Poultry (Eggs and Tissues).**

Matrix Metabolite/ fraction	Egg white Day 4 (0.362 ppm)	Egg yolk Day 4 (0.186 ppm)	Liver (0.210 ppm)	Muscle (0.634 ppm)	Fat (0.336 ppm)	Skin (0.309 ppm)
Percent TRR in matrix (ppm in parentheses)						
Pirimiphos-methyl	3.4 (0.012)	12.1 (0.023)	1.52 (0.003)	3.9 (0.025)	85.4 (0.287)	31.0 (0.095)
Compound A	0.7 (0.003)	ND <sup>a</sup>	1.21 (0.003)	1.0 (0.006)	6.0 (0.019)	3.1 (0.010)
Compound C	0.7 (0.003)	4.5 (0.009)	0.83 (0.002)	3.1 (0.020)	ND	25.2 (0.078)
Compound D	1.4 (0.005)	22.1 (0.041)	14.2 (0.030)	45.2 (0.287)	ND	1.4 (0.004)
Compound E	ND	ND	ND	ND	10.9 (0.033)	ND
Compound F	3.1 (0.011)	ND	ND	5.7 (0.036)	ND	ND
Compounds E/F <sup>b</sup>	63.2 (0.229)	7.3 (0.014)	12.7 (0.027)	25.6 (0.162)	ND	3.4 (0.011)
Total Identified	72.5 (0.263)	46.0 (0.087)	30.46 (0.065)	84.5 (0.536)	102.3 (0.339)	64.1 (0.198)
HPLC unknowns <sup>c</sup>	21.3 (0.077)	1.0 (0.002)	22.7 (0.047)	4.2 (0.026)	None	21.0 (0.065)
Other <sup>d</sup>	1.65 (0.006)	14.6 (0.027)	40.4 <sup>e</sup> (0.085)	3.52 (0.022)	8.63 (0.029)	7.82 (0.024)
Total Partially Characterized	22.95 (0.083)	15.6 (0.029)	63.1 (0.132)	7.72 (0.048)	8.63 (0.029)	28.82 (0.089)
Total Identified + Partially Characterized	95.6 (0.346)	61.4 (0.116)	93.6 (0.197)	92.2 (0.584)	111 (0.339)	92.9 (0.287)

<sup>a</sup> ND = not detected

<sup>b</sup> Includes Compounds E and F not resolved by HPLC; does not include isolated Compounds E and F, which were from different fractions.

<sup>c</sup> Total of unknown HPLC peaks/areas each at <5% of TRR.

<sup>d</sup> Total of numerous fractions, each considered characterized to some extent and accounting for <10% of TRR and/or <0.05 ppm of radioactivity.

<sup>e</sup> Includes the final insoluble fraction, which accounted for 32.9% of TRR and 0.069 ppm after mild and rigorous acid hydrolysis.

**Processed Commodities:**

In seven trials, stored corn grain received five 1X applications at 60- to 90-day intervals over a period of 270 days. Samples were collected 0-90 days after each application. The highest residue of 336 samples was 18.8 ppm and the highest average field trial (HAFT) residue value was 12.7 ppm. The des-ethyl metabolite R36341 was a minor component of the residue at  $\leq 0.08$  ppm. In seven trials, stored sorghum grain received five 1X applications at 60- to 90-day intervals over a period of 270 days. Samples were collected 0-90 days after each application. The highest residue was 18.2 ppm and the HAFT was 17.2 ppm.

Results of corn, sorghum and wheat processing studies are the following:

Residues concentrated at an average of 12.2x in refined corn oil and 3.8x in aspirated grain fractions of corn; residues did not concentrate in other milled fractions.

Residues concentrated in aspirated grain fractions of sorghum at 2.3x. Residues did not concentrate in sorghum flour.

Combined residues in wheat grain were 29.5 ppm. Residues concentrated by a factor of 2.1x in wheat bran and by a factor of 3.4x in aspirated grain fractions of wheat. Residues did not concentrate in wheat flour.

Attachment: Section G of PP#6F4740

cc: J. Stokes (CEB2); R.F.; MARC F.; PP#6F4740

RDI:SHummel:04/13/98

7509:CEB2:CM#2:Rm803:JStokes:js:305-7561:04/13/98

ATTACHMENT

Section G - Pirimiphos-methyl  
Reasonable Grounds in Support of the Petition

After evaluation of data, Wilbur-Ellis is proposing that the tolerance expression for pirimiphos-methyl include only the parent compound and its desethyl metabolite (Compound A, empirical formula  $C_9H_{16}N_3O_3PS$ ) for the following reasons:

- (1) The hydroxypyrimidine minor metabolites were required by the EPA to be included in the original 1978 tolerance expression due to a lack of metabolism data at that time, not because of inherent properties of the metabolites themselves.
- (2) Metabolism and feeding studies since that time have shown that there are four principal metabolites. Of those metabolites, only Compound A is phosphorus-containing and therefore a potential ChE inhibitor.
- (3) The Codex Alimentarius has expressed pirimiphos-methyl tolerances only in terms of the parent compound and its phosphorus-containing metabolites. Removal of the hydroxypyrimidines would bring EPA tolerances closer to harmony with the Codex.
- (4) EPA Dietary Exposure Branch has stated that they are only concerned with the phosphorus-containing metabolite(s), which are potential ChE inhibitors.
- (5) The hydroxypyrimidine metabolites are only produced at very low levels in plants and animals treated with/fed pirimiphos-methyl and are rapidly excreted as non-toxic products.
- (6) The structure of the hydroxypyrimidines indicates no reason for toxicological, carcinogenic or teratogenic concerns.

Background of the Compound

Pirimiphos-methyl is an organophosphate (OP) insecticide of generally low toxicity. Based on acute toxicity studies in a variety of species it can be classified as slightly toxic to practically nontoxic (Klaassen 1986). Pirimiphos-methyl was first introduced in England in 1970 and was registered in the United Kingdom and Europe under the brand names Actellic® and Blex®.

In 1978, the registration of pirimiphos-methyl was sought in the United States. Upon review of the existing studies with wheat and rice (MRID 00130402), rats (MRID 00080737, 00080738, 00080739, 00129345, and 43650801) and dogs (MRID 00080737 and 00080738), EPA concluded that the metabolism data was inadequate. In 1979, the Agency included the hydroxypyrimidine metabolites (Compounds C, D, and E in Table 1 below) in the tolerance expression due to limited plant and animal metabolism data and magnitude of the residue feeding information.

In 1992, ownership of the Actellic 5E registration was transferred from ICI Americas, Inc. (with registration number 10182-79) to Wilbur-Ellis Company (current registration number 2935-486). Wilbur-Ellis Company is supporting the existing uses of pirimiphos-

methyl under reregistration. New metabolism studies were conducted with pirimiphos-methyl in corn (MRID 42903501), laying hen chickens (MRID 42903503), and lactating goats (MRID 42903502). Magnitude of the residue feeding studies in lactating cows and in hens have recently been completed and will be submitted to EPA by June 30, 1996.

In magnitude of residue studies for both animal and plant matrices, two separate analytical methods are required: one method for pirimiphos-methyl and Compound A, and a separate method for the three hydroxypyrimidine metabolites.

#### Identification of the Metabolites

The metabolism studies have shown that pirimiphos-methyl has four principal metabolites (see Table 1). The oxygen analog, part of the Codex Alimentarius tolerance expression (see below), has not been detected in recent metabolism studies. If it does exist, it is believed that it would be a briefly-occurring intermediate during hepatic transformation of pirimiphos-methyl to Compound A.

The four principal metabolites are:

1. O-(2-ethylamino-6-methyl-pyrimidin-4-yl) O,O-dimethylphosphorothioate (Compound A, "desethyl", empirical formula  $C_9H_{16}N_3O_3PS$ )
2. 2-diethylamino-6-methyl-pyrimidin-4-ol (Compound C, empirical formula  $C_9H_{15}N_3O$ )
3. 2-ethylamino-6-methyl-pyrimidin-4-ol (Compound D, empirical formula  $C_7H_{12}N_3O$ )
4. 2-amino-6-methyl-pyrimidin-4-ol (Compound E, empirical formula  $C_5H_7N_3O$ ).

Compound A ("p-m desethyl"), the only phosphorus-containing metabolite, is the only metabolite of toxicological significance since only Compound A (and the parent pirimiphos-methyl) have the potential for inhibiting cholinesterase.

#### Toxicological Considerations

Organophosphate insecticides exert their toxic effect through the inhibition of the cholinesterase (ChE) enzymes, particularly acetylcholinesterase (AChE) in the central nervous system (O'Brien 1967; Carlton 1969; Eaton and Klaassen 1996; Murphy 1986; Zinkl et al. 1979). Therefore, the concern with toxicity of residues from application of organophosphates, such as pirimiphos-methyl, is associated with the parent compound and any metabolites that are cholinesterase inhibitors.

Tolerances for pirimiphos-methyl have been established since the 1970's in the Codex Alimentarius as the combined residues of pirimiphos-methyl, its oxygen analogue, and the metabolite N-desethyl-pirimiphos-methyl (Compound A). This remains the case to the present day. Removal of the hydroxypyrimidine metabolites from the EPA tolerance expression will therefore bring EPA tolerances into closer harmony with international tolerances.

Although the EPA originally required inclusion of hydroxypyrimidine metabolites in the tolerance expression, the Toxicology Branch has since determined that the hydroxypyrimidine metabolites are not of toxicological significance. In evaluating proposed tolerances of pirimiphos-methyl, EPA (S. H. Willett, Dietary Exposure Branch, Health

Effects Division, March 23, 1990 memo) states the following:

"Since the adverse health effects involve cholinesterase inhibiting residues only, the dietary exposure assessment should be done based on levels of the parent compound (see also memo of S. Willett, 2/28/90)."

On behalf of Wilbur-Ellis, two independent evaluations of the toxicological significance of pirimiphos-methyl metabolites were recently conducted. Both reviewing scientists, Dr. Gino Marco, Marco-Tech, and Dr. Peter Ryle, Huntingdon Life Sciences, concluded that the hydroxypyrimidine metabolites (Compounds C, D, and E) of pirimiphos-methyl should have no toxicological significance and should pose no problem upon ingestion. These compounds are rapidly excreted from the body and are not cholinesterase inhibitors.

Dr. Marco (Attachment 3) reviewed earlier and recent goat, hen, and stored grain metabolism studies, as well as earlier rat and dog feeding studies. He characterized the hydroxypyrimidine production in plants as minor. He found that pirimiphos-methyl is rapidly degraded in animals and that its resultant metabolites, particularly the hydroxypyrimidine metabolites, are subsequently degraded and/or excreted without accumulation.

Dr. Ryle (Attachment 4) approached the question of toxicological significance of the hydroxypyrimidines from a consideration of molecular structure and characteristics of similar related molecules. His paper concludes that toxicological, carcinogenic or teratogenic problems would be unlikely with these three compounds.

For the above reasons, Wilbur-Ellis is proposing that the pirimiphos-methyl tolerance expression be amended as detailed in Section F of this petition.



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